gland-free meals. These results are in accordance with those reported in this paper.

Summary

A biological study was made of the protein values of three cottonseed meals and one cottonseed flour as influenced by different methods of processing, using the albino rat as the experimental animal. The protein efficiency of these meals and flour was investigated by the rat growth method at 10, 8, and 5% levels of protein intake. The best product from the standpoint of protein efficiency was the one which was solvent-extracted and had no heat treatment. The cottonseed flour which had the most drastic heat treatment showed the poorest protein efficiency. The biological value of the proteins in this product was improved by the addition of lysine and methionine, which had been either partially destroyed or made unavailable during processing.

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AMINO ACIDS
In Cane Juice and Cane Final Molasses

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Cane blackstrap molasses contains as much as 50 to 60% simple sugars. Recovery of these sugars in sucrose production is not economical because of the presence of substances formed from reaction of the sugars with amino acids of sugar cane juice. Chromatographic procedures were applied to this juice and its corresponding molasses to identify the responsible amino acids. Two-dimensional, ascending paper chromatography indicates the probable presence of asparagine, aspartic acid, glutamine, glutamic acid, glycine, alanine, valine, leucine (or isoleucine), serine, tyrosine, and γ-aminobutyric acid in Florida cane juice and (except serine, tyrosine, and glutamine) in its corresponding final molasses.

CANE JUICE is a major commercial source of sucrose, yet a very significant amount of this sugar remains in blackstrap molasses, a by-product of sucrose production. As a part of the program on the composition of molasses being conducted in this laboratory, assays of cane juice and the final or blackstrap molasses produced therefrom were undertaken to determine their component amino acids, which are considered significant in molasses formation (*16*).

By means of the difficult older isolation techniques, Zerban (*15*) isolated and identified L(*levo*)-asparagine, (*dextro*)-glutamine, and tyrosine in Puerto Rican cane juice. L-Asparagine was the most abundant component. Several other reported demonstrations of amino acid in cane juice, such as that of Maxwell (*9*) for asparagine, cannot be considered experimentally adequate. In Hawaiian cane final molasses, Payne (*10*) reports the presence of aspartic acid, glutamic acid, pyrrolidone carboxylic acid (a probable alteration product of glutamic acid), and lysine. The free amino acid content of Jamaican cane juice (*11*, *13*) and of beet molasses (*6–8*) has been investigated by paper chromatographic methods.

In the present work the authors have devised procedures whereby the amino acid fraction of cane final molasses could be separated sufficiently free of contaminants to enable the use of the techniques of paper chromatography. The amino acid content of the cane juice from which the final molasses originated was determined by essentially the same general methods, in order to learn the fate of these substances in the final cane molasses.

Cane Juice

The unclarified sugar cane crusher juice was collected at Clewiston, Fla., on January 6, 1951. It was quick-frozen, packed in solid carbon dioxide, and transported to Columbus, Ohio, where it arrived still frozen. For partial clarification the thawed juice (200 grams) was poured on a bed of Celite No. 545 (15 grams, Johns-Manville Co., New York, N. Y.) in a jacketed 4-inch Büchner funnel with water at 4° to 6° passing through the jacket. The filtrate was slightly turbid.

Analysis (% original juice). Solids, 14.1; sucrose, 10.7; reducing sugars (as invert sugar), 1.3; pH, 5.7; D_4^{25} 1.052; nitrogen (Kjeldahl), 0.041.

Cane Juice Cations An amount of 500 grams of partially clarified cane juice was added at the top of a 480 × 32 mm. (diameter) column (dimensions refer to the packing) of Amberlite IR-120 (Rohm & Haas Co., Philadelphia, Pa.) The column was backwashed and washed with distilled water until the effluent was free of sugars. The cane juice cations were recovered from the Amberlite IR-120 with 370 ml. of 10% hydrochloric acid followed by 350 ml. of water. This solution was concentrated under reduced pressure at 48° to 50° to 200 ml. for paper chromatography. Kjeldahl nitrogen was found to be 0.031% (basis original juice; 75% of the nitrogen originally present).

Separation of Amino Acid Fraction of Cane Juice by Fuller's Earth Clay An amount of 450 grams of partially clarified cane juice was dewatered by ice sublimation; yield was 64.11 grams.

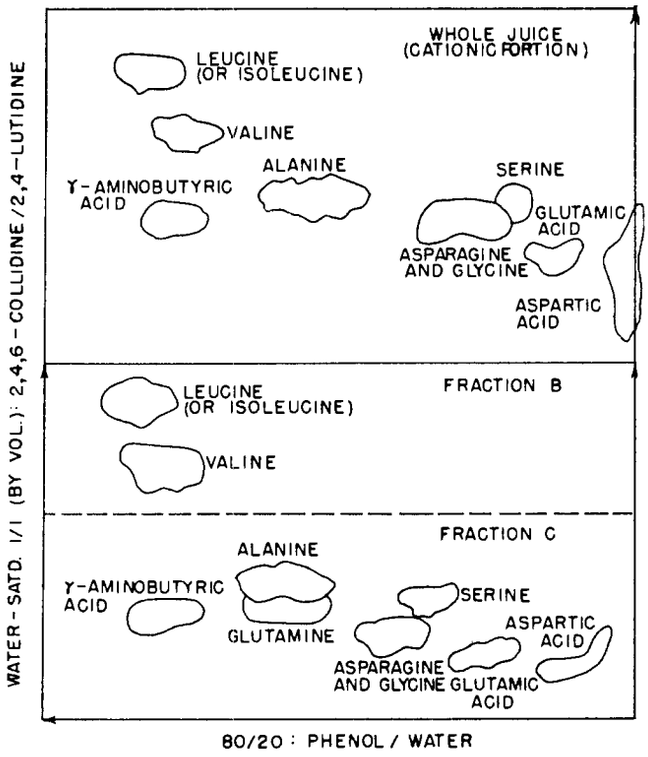

Figure 1. Amino Acids in Cane Juice

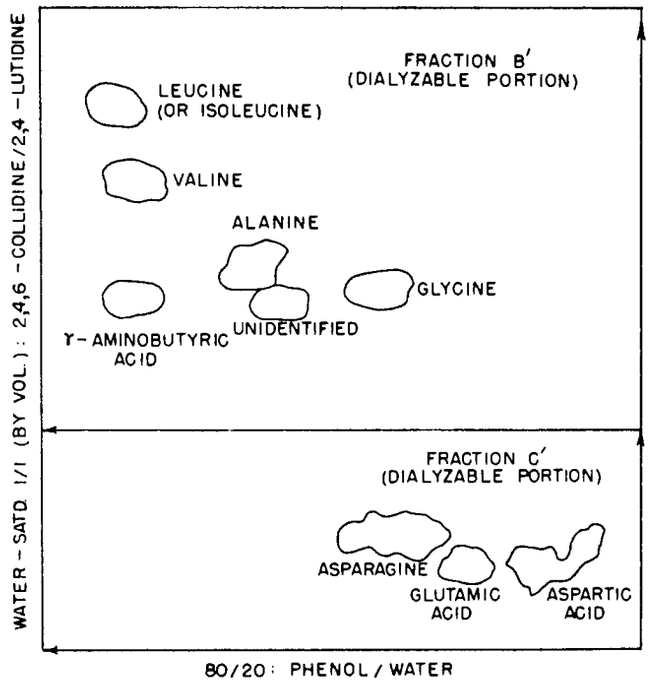

Figure 2. Amino Acids in Cane Final Molasses

These solids were dissolved in 30 ml. of water and diluted with 100 ml. of absolute methanol; the resulting solution was added slowly to an agitated mixture of 25 grams of Celite and 900 ml. of absolute methanol. The suspension was poured on a 11 to 12 × 7 to 9 cm. (diameter) column of fuller's earth clay (Floridin Co., Warren, Pa.)/Celite (5 to 1, weight ratios), prewet with 1400 ml. of 95% ethyl alcohol (the azeotrope), in a 2-liter pharmaceutical percolator connected to a dropping funnel for the collection of effluent fractions. The chromatogram was developed successively with 5 liters of 95 ethyl alcohol–5 water, 80 ethyl alcohol–20 water, 50 ethyl alcohol–50 water (volume ratios before mixing), and water. The residue from the 95 ethyl alcohol–5 water column effluent (fraction S) was treated to remove 86% (41.4 grams) of the available sucrose by crystallization (*1*) to leave a palatable, straw-yellow sirup; yield was 7.52 grams (fraction A). A palatable amber sirup was obtained from the 80 ethyl alcohol–20 water effluent after solvent removal; yield was 7.76 grams (fraction B). The residue from the 50 ethyl alcohol–50 waters and water fractions were tan, amorphous solids. Yields were, respectively, 1.40 grams (fraction C) and 2.30 grams (fraction D); total return was 60.3 grams (95% of determined solids).

Analysis. Nitrogen (Kjeldahl). Fraction A, 0.16; fraction B, 0.18; fraction C, 10.46; fraction D, 0.63.

Cane Blackstrap Molasses

The blackstrap molasses was collected in the mill at Clewiston, Fla., on January 6, 1951, 12 hours after the cane juice was obtained.

Analysis (% original molasses). Nitrogen (Kjeldahl), 1.37; solids, 78.0.

Separation of Amino Acid Fraction of Cane Final Molasses An amount of 100 grams of Florida blackstrap molasses was diluted with 30 ml. of water and a smooth paste prepared by the addition of 50 grams of a mixture of fuller's earth clay–Celite (5 to 1). This paste was suspended in 1500 ml. of absolute ethyl alcohol and the mixture was fractionated on a column of fuller's earth clay–Celite by the procedure described above for the cane juice. The residue yields of the column effluents after development with 95/5, 80/20, and 50/50 ethyl alcohol–water and water were 51.5 grams (fraction A′), 8.7 grams (fraction B′), 10.8 grams (fraction C′), and 5.8 grams (fraction D′), respectively. The physical appearances of these fractions have been described (*2*).

Analysis. Nitrogen (Kjeldahl). Fraction A′, 0.21; fraction B′, 5.06; fraction C′, 5.83; fraction D′, 1.72.

An aqueous solution of each of fractions B′ (1.74%), C′ (1.08%), and D′ (0.58%) was added to a cellophane bag (made of osmosis membrane 70160-C, a product of the Central Scientific Co., Chicago, Ill.) and was dialyzed against distilled water for 3 days. The dialyzed solutions did not give the ninhydrin test; the solutions of dialyzable substances (passing through the membrane) showed a positive ninhydrin reaction.

Amino Acid Chromatography

For the qualitative detection of the amino acids, the authors utilized two-dimensional, ascending paper chromatography, following the general technique of Williams and Kirby (*14*) and employing the developers described by Consden, Gordon, and Martin (*3*) as modified by Dent (*5*). Final identification of all spots (except those for γ-aminobutyric acid and tyrosine) was effected by spot enhancement with known substances.

A 5-ml. aliquot of the cane juice cation solution was concentrated under reduced pressure at 50° C. to 2 to 3 ml. and crystals (identified as potassium chloride) were formed. The mother liquor was removed by decantation and the evaporation was continued until the solvents were removed. Five milliliters of water was added to the residue and the evaporation under reduced pressure was repeated. This process was repeated once again to decrease hydrochloric acid further. The final residue was dissolved in 2 ml. of water. This solution was added with a micropipet a drop at a time to the same point 2 cm. from each edge of the lower right-hand corner of an 18.5-cm. square of Whatman No. 1 filter paper (some experiments were made with a 40-cm. square). After exposure to ammonia vapor, each spot was allowed to dry a few seconds at 80° to 90°C. before the next drop was added. This process was repeated until the amount of deposited material was sufficient to produce a suitable chromatogram.

The edges of the paper perpendicular to the machine direction of the paper were fastened with staples, so that the edges of the paper did not quite touch. This paper cylinder with the adsorbate

near the bottom was allowed to stand in a reservoir 3 to 4 mm. deep of 80% phenol (20% water) in a tightly sealed glass chamber. The cylinder was removed from the chamber when the solvent front reached the top and was allowed to dry in air at room temperature overnight and finally for 5 minutes at 80° to 90°. The opposite edges of the paper were fastened as described previously. The paper cylinder with the adsorbate near the bottom was placed in a reservoir, 3 to 4 mm. deep, of 1-1 2,4,6-collidine–2,4-lutidine (volume ratios) (mixture saturated with water at 25°) in a tightly sealed chamber. The cylinder was again removed when the solvent front reached the top and, with the staples removed, was allowed to dry in air at room temperature. The chromatogram was dried at 90° to 95° for 10 minutes and was sprayed as uniformly as possible with 0.25% ninhydrin in 1-butanol saturated with water. The color spots were usually allowed to form at room temperature (quicker color development could be obtained at 90° to 95°).

The results of the two-dimensional chromatogram are shown in Figure 1. There was some difficulty in interpreting the chromatogram in the aspartic acid–glycine region because of the distortion caused by adsorbate acidity and the presence of inorganic salts. Overlapping and distortion of asparagine with glycine were considerable, but the orange yellow color of the asparagine spot was of aid. On treatment of this cation fraction with 6 *N* hydrochloric acid in a sealed tube at 95° for 40 hours, the asparagine spot disappeared, by hydrolysis to aspartic acid, and the glycine spot was then clearly evident. Aspartic acid occurred in very high concentration, perhaps exceeding in color intensity those of the remaining spots combined. Alanine was next in color intensity, followed by serine and glutamic acid. Glycine, γ-aminobutyric acid (methionine sulfoxide eliminated by hydrogen peroxide test), and valine showed weak spots with leucine (or isoleucine) even weaker.

An aqueous solution of fractions B and C from cane juice was added to the filter paper squares and chromatographed as described above, except that the ammonia treatment was not required. The results are shown in Figure 1. The most prominent spot was that of asparagine, followed by strong spots from alanine and glutamic acid; serine gave a slightly weaker spot. γ-Aminobutyric acid, glutamine, and aspartic acid followed in intensity, while valine and leucine (or isoleucine) showed very weak spots. Glycine was obscured by asparagine. Fractions A and D were similarly investigated but showed no significant amounts of ninhydrin staining substances.

The solids from 135 ml. of cane juice cation eluant, dissolved in 4 ml. of water, were placed at the top of a 54 × 3.2 cm. (diameter) powdered cellulose column (prewetted with the developer) and developed with 750 ml. of water-saturated 1-butanol, the last 150 ml. of which was subjected to paper chromatography, thereby revealing an isolated grayish blue spot in the position characteristic for tyrosine

Two-dimensional paper chromatograms of fractions B′ and C′ from the cane final molasses were prepared as described for fractions B and C; fractions A′ and D′ did not yield spots on chromatography. The results are shown in Figure 2. Again the most intense spot was asparagine, followed by strong spots of alanine, glycine, and aspartic acid. Spots for glutamic acid, γ-aminobutyric acid, valine, and leucine (or isoleucine) were less intense. An unknown spot was located below and to the right of alanine.

Discussion

The probable presence in the cane juice of aspartic acid, asparagine, glutamine, glutamic acid, glycine, alanine, valine, leucine (or isoleucine), serine, tyrosine, and γ-aminobutyric acid is indicated by paper chromatography. The authors' work on cane juice was completed before the report of Wiggins and associates (*11*, *13*) was brought to their attention. The results of the two laboratories are in essential agreement, except that Pratt and Wiggins found no tyrosine and reported a weak spot for lysine. A concentration of the cane juice cations on a cellulose column (*4*, *12*) was found by the authors to be required for the demonstration of the presence of tyrosine by paper chromatography. This amino acid is present in low amount and is difficult for detection by these methods. The basic amino acid lysine was not detected in the author's work.

Pratt and Wiggins (*11*) state that the application of paper chromatography directly to the whole cane juice yielded unsatisfactory results. Improvement was obtained by them when the amino acid fraction was first separated by adsorption on a cationic exchange resin with subsequent elution by hydrochloric acid. The present authors found that this still yielded a heavy inorganic salt background in the paper chromatography and effected considerable hydrolysis of the amides present. It was unsatisfactory for the detection of the amino acids in cane final molasses. For this purpose they resorted to a preliminary separation of the amino acids from the molasses into two fractions (B′ and C′) by successive adsorption and elution on fuller's earth clay, followed by a dialysis through a cellulose membrane. Good paper chromatograms were then obtained.

When this procedure, with the dialysis omitted, was applied to whole cane juice, chromatograms were obtained which were of enhanced quality and showed less distortion of spots than when the same techniques were applied to the cationic fraction separated by an ion exchange resin (Figure 1). Fraction B from the cane juice contained valine and leucine (or isoleucine); fraction C (of the cane juice) contained, in addition to glutamine and an additional amount of asparagine, the remainder of the amino acids found in the cane juice cations. The cation exchange resin had effected considerable hydrolysis of the amide functions in asparagine and glutamine. Thus, in the cane juice, asparagine is predominant, followed by alanine, glutamic acid, and serine; weak spots were exhibited by valine, leucine (or isoleucine), γ-aminobutyric acid, glutamine, and glycine.

The dialyzable portions of fractions B′ and C′ from the final molasses were subjected to paper chromatographic assay and yielded the results diagrammed in Figure 2. The distribution between the two fractions was different from that observed with cane juice (Figure 1), as the presence of other substances in the molasses allowed a more ready elution of the adsorbate from the clay. Spots representing glycine, alanine, valine, leucine (or isoleucine), γ-aminobutyric acid, and an unknown substance were found in the dialyzable portion of fraction B′; the corresponding portion of fraction C′ contained only aspartic acid, asparagine, and glutamic acid. Serine, tyrosine, and glutamine were present in the cane juice but were not found in the molasses.

These results, together with the less complete data of Payne (*10*), show that the amino acids of the cane juice survive in large part in the final molasses. This is a surprising finding and is probably due to the inert diluent effect exercised by the sucrose molecules which come between and largely prevent the "browning" or interaction of the reducing sugars with the amino acids. In beet sugar molasses, Mariani and Torraca (*6–8*) record the acids found by the authors in cane juice and the following in addition, as present in small amounts: threonine, phenylalanine, proline, and arginine.

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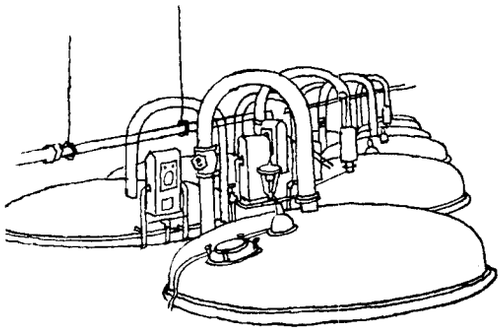

Fermentation and Its Products Have Much to Offer

FUNGAL SACCHARIFYING AGENTS

Amylolytic Factors of Bran Culture and Submerged Culture

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Three saccharifying mold strains have been cultivated on bran and in submerged culture. At optimum levels the alcohol yields from corn mashes saccharified by submerged culture and mold bran from *Aspergillus niger* NRRL 330 were, respectively, 5.44 and 5.38 proof gallons per standard bushel, from *A. niger* NRRL 337 5.28 and 5.30 proof gallons, and from *A. oryzae* ISC 38b 5.13 and 5.28 proof gallons per bushel. Analysis showed submerged and bran cultures of *A. niger* 330 were highest in maltase activity and lowest (very low) in α-amylase. Cultures of *A. oryzae* 38b were highest (very high) in α-amylase and lowest in maltase and limit dextrinase. Cultures of *A. niger* 337 were highest in limit dextrinase and intermediate (high) in α-amylase and maltase. Slight correlation seemed to exist between alcohol yields and maltase activity, but there was no correlation for the other enzymes. However, the optimum level of a saccharifying agent for maximum alcohol yield cannot be predicted from the maltase activity. Although the short fermentation test method of Reese, Fulmer, and Underkofler is applicable for determining the optimum levels of similar fungal saccharifying preparations from the same mold strain, it cannot be used to compare preparations produced by different mold strains nor by the same strain cultivated in different manner.

USE OF FUNGAL saccharifying agents for conversion of starchy mashes for alcoholic fermentation has received much attention during the past two decades. Two successful procedures for cultivating molds to produce active saccharifying agents have been developed. The first was the growth of a selected strain of the mold *Aspergillus oryzae* on moistened wheat bran (*11*). The resulting mold bran may be used in the same manner as malt for conversion of fermentation mashes. More recently amylase-producing molds have been cultivated in submerged culture with vigorous agitation and aeration (*1, 3, 6*).

The media employed for submerged culture have generally been mixtures of thin stillage or distillers' dried solubles and corn, and in most cases the culture employed has been *Aspergillus niger* 337 of the Northern Regional Research Laboratory. The submerged culture preparation is utilized by mixing approxi-